Molecular Biology Select

Cell

Although genomic rearrangements can have detrimental consequences, they are intentionally triggered in certain contexts such as nuclear maturation and immune evasion in protozoa, adaptation to environmental stress in bacteria, and immune system development in vertebrates. This Molecular Biology Select describes recent advances in the understanding of genomic rearrangements that benefit an organism.

Transposases Take Center Stage in a Nuclear Makeover

Scanning electron micrograph of two mating *Oxytricha* cells. Image courtesy of R. Hammersmith.

Genomic rearrangements are used by the ciliated protozoan *Oxytricha trifallax* to discard 95% of its germline genome after sexual conjugation. The ciliate then stitches together the remaining DNA to form a new gene-dense somatic genome that is remarkably consistent from cell to cell. A new report from Nowacki and colleagues (2009) suggests that transposases, enzymes capable of cutting and joining DNA, are critical to this process. The *O. trifallax* germline genome harbors thousands of transposable DNA elements, each containing a gene encoding one of three closely related transposases that are necessary for transposon mobility. The authors observe that the transposase genes are only expressed in cells during conjugation. Speculating that these enzymes may play a role in directing genome reorganization, the authors disrupt the expression of all three types of transposase genes at a later stage of conjugation after mating cells separate. The loss of transposase expression not only results in decreased cell survival and delayed cell growth, but also affects the processing of germline DNA to somatic DNA. These cells still contain precursor genomic DNA that has not undergone rearrangements or has undergone incomplete or aberrant rearrangements at specific loci. They also accumulate longer unprocessed chromosomes and retain transposase genes. Thus, these findings suggest that this ciliate may have co-opted its parasitic DNA elements to facilitate precise excision and recombination in the creation of a new genome.

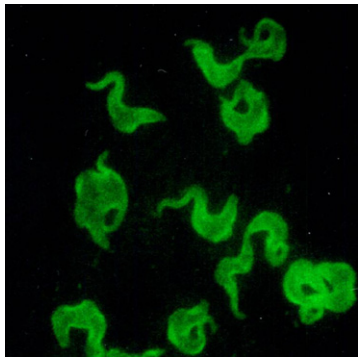
M. Nowacki et al. (2009). Science ***324****, 935–938*.

A Hop, Skip, and a Jump to Genetic Diversity

Genomic rearrangements resulting from the random transposition of mobile DNA elements can alter or disrupt gene expression. Coros and colleagues (2009) now propose that the bacterium *Escherichia coli* takes advantage of genome rearrangements to adapt to metabolic stress. They show that *E. coli* amplifies genetic diversity in response to nutrient starvation by mobilizing retrotransposon-like DNA elements called group II introns, which can integrate into DNA randomly or at specific sequences. The authors find that the ability of group II introns to undergo integration is regulated by cyclic AMP (cAMP) or guanosine tetraphosphate (ppGpp). These small molecules tune global gene expression according to the cell's nutritional state. Glucose deprivation induces cAMP-mediated regulation of gene expression and promotes group II intron movement. In cells with impaired cAMP biosynthesis, this retromobility is diminished, but can be restored by the addition of cAMP. Likewise, amino acid starvation elevates the abundance of ppGpp and increases the retromobility of group II introns. Thus, increased cAMP or ppGpp levels promote general retromobility of the group II introns. Strikingly, both cAMP and ppGpp appear to favor random integration of group II introns over their integration at specific sites. Although the mechanism by which these small molecules drive the movement of group II introns remains to be elucidated, the findings of Coros et al. provide intriguing evidence for a regulated process of mutagenesis in bacteria to generate increased genetic diversity during times of stress.

C.J. Coros et al. (2009). Mol. Cell. ***34****, 250–256.*

Breaking into Immune System Evasion



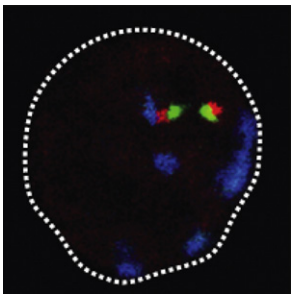
T. brucei stained with an antibody against a Variant Surface Glycoprotein (green). Image courtesy of G.A.M. Cross.

Altering gene expression by genomic rearrangements underlies the ability of the parasitic protozoan *Trypanosoma brucei* to evade detection by the host immune system. In a process termed antigenic variation, *T. brucei* switches its variant surface glycoprotein (VSG) coat. In a new study, Boothroyd and colleagues (2009) investigate the molecular events behind this switch. *T. brucei* harbors thousands of different *VSG* genes, but only one of these is expressed from a specialized site in the genome. During antigenic variation, the *VSG* gene at the expressed locus is swapped for another. This commonly occurs through homologous recombination, which might be initiated by a double-stranded break (DSB). To test this hypothesis, the authors use an exogenous endonuclease to induce DSBs at specific sites in the genome of *T. brucei*. All *VSG* genes harbor an upstream 70 bp repeat sequence. Remarkably, the induction of a DSB adjacent to the 70 bp repeat sequence upstream of the expressed *VSG* gene—and not elsewhere in the genome—causes an ~250-fold increase in the rate of antigenic switching relative to

control strains. Notably, the elevated levels of *VSG* switching require the presence of the 70 bp repeat sequence, which likely provides homology for recombination during the gene conversion event. Boothroyd and colleagues further show that DSBs occur naturally within the 70 bp repeat sequence near the expressed *VSG* locus in wild-type *T. brucei* strains. In contrast, no such breaks are observed in the untranscribed 70 bp repeat sequences associated with silenced *VSG* genes elsewhere in the genome. The authors propose that DSBs could spontaneously arise from transcription through structurally unstable repetitive motifs in the 70 bp repeat region of the expressed *VSG* locus. Thus, they provide compelling evidence that the DSB is a primary trigger of this immune system evasion tactic in *T. brucei*, establishing a basis for future work exploring the regulation of this process.

C.E. Boothroyd et al. (2009). Nature. ***459****, 278–281.*

Knowing When to Hold 'em and When to Cleave 'em

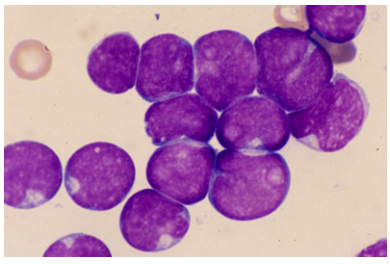


After RAG-mediated cleavage, paired immunoglobulin alleles (red and green) in a developing B cell differentially localize relative to pericentromeric heterochromatin (cyan). Reprinted with permission from Macmillan Publishers Ltd: Nature Immunology (S. Hewitt et al.), copyright 2009.

In vertebrates, developing T and B cells undergo V(D)J recombination to assemble a broad repertoire of different antigen receptor genes by rearranging gene segments arrayed along the T cell receptor and immunoglobulin loci. Because it is critical that each lymphocyte expresses only a single type of antigen receptor, recombination is completed on only one of two homologous alleles through a poorly understood process known as allelic exclusion. Hewitt and colleagues (2009) now show that coordination of V(D)J recombination between homologous alleles of two B cell antigen receptor (immunoglobulin) genes is regulated by the V(D)J recombinase component RAG-1 and the DNA damage checkpoint protein ATM. The authors observe that in a large percentage of developing primary B cells isolated from mouse bone marrow, homologous alleles of the immunoglobulin genes come together to form a pair. Pairs of unrecombined alleles are frequently found in the region of the nucleus containing euchromatic DNA. However, paired alleles in the process of V(D)J recombination are positioned such that the unrearranged allele is held within pericentromeric heterochromatin and the rearranged allele is positioned within euchromatin. Speculating that this differential localization of alleles underlies the allelic exclusion process, the authors show that recruitment of one allele to pericentromeric heterochromatin requires the DNA cleavage activity of RAG-1 and the presence of the ATM protein. From these data, the authors propose that RAG-1 differentially "marks" one of the alleles by introducing a DNA break, which induces ATM to reposition the uncleaved allele to the heterochromatic nuclear region to shield it from rearrangement. ATM also appears to limit RAG-1 cleavage to a single allele, further ensuring genomic stability and proper B cell development. Given that ATM is activated by the cleaved allele, one intriguing question for future studies is how it is able to drive, in *trans*, the relocalization of the uncleaved allele to pericentromeric heterochromatin.

S. Hewitt et al. (2009). Nat. Immunol. ***10****, 655–664.*

A Life after the PHD for H3K4 Methylation



Human pre-T acute lymphoblastic leukemia cells that harbor chromosomal rearrangments. Image courtesy of R.K. Brynes.

Sites of V(D)J recombination in mammalian lymphocytes are marked by trimethylation of lysine 4 in histone H3 (H3K4me3). This histone modification has been thought to solely function in recruiting the RAG recombinase complex through its interaction with the plant homeodomain (PHD) finger of RAG-2. Shimazaki and colleagues (2009) now report evidence that H3K4me3 is more than a passive binding platform for the RAG complex. During V(D)J recombination, the RAG complex accomplishes DNA cleavage by nicking the DNA at recombination signal sequences and catalyzing the formation of two DNA hairpins from the broken DNA ends. To investigate the role of H3K4me3 in this process, the authors examine the effect of H3K4me3 on the enzymatic activity of the mouse RAG complex in vitro. They incubate purified RAG complex with histone H3 tail peptides that are trimethylated at K4 and linked to DNA containing a recombination signal sequence. Remarkably, the presence of tethered H3K4me3 peptides stimulates both steps of RAG catalytic activity, supporting an active function for H3K4me3 in V(D)J recombination. Free H3K4me3 peptide similarly stimulates RAG activity, indicating that this newfound function for H3K4me3 is independent of its known role in tethering the RAG complex to DNA. Intriguingly, H3K4me3 also promotes in vitro the RAG-mediated processing of weak cryptic recombination signal sequences that are associated with SCL-SIL chromosomal rearrangements in T-cell acute lymphoblastic leukemia. Given that certain chromosomal breakpoints associated with subtypes of lymphoma and leukemia lie within regions of H3K4me3-modified chromatin, the authors propose that erroneous stimulation of RAG activity at these sites may contribute to the inception of these malignancies.

N. Shimazaki et al. (2009). Mol. Cell. ***34****, 535–544.*

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